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Brief Research Communication

Characterization of Six Mutations in Exon 37 of Neurofibromatosis Type 1 Gene

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Neurofibromatosis type 1 (NF1) is one of the most common inherited disorders, with an incidence of 1 in 3,000. We screened a total of 320 unrelated NF1 patients for mutations in exon 37 of the NF1 gene. Six independent mutations were identified, of which three are novel, and these include a recurrent nonsense mutation identified in 2 unrelated patients at codon 2281 (G2281X), a 1-bp insertion (6791 ins A) resulting in a change of TAG (tyrosine) to a TAA (stop codon), and a 3-bp deletion (6839 del TAC) which generated a frameshift. Another recurrent nonsense mutation, Y2264X, which was detected in 2 unrelated patients in this study, was also previously reported in 2 NF1 individuals. All the mutations were identified within a contiguous 49-bp sequence. Further studies are warranted to support the notion that this region of the gene contains highly mutable sequences.

KEY WORDS: neurofibromatosis type 1, mutations, exon 37

INTRODUCTION

Von Recklinghausen neurofibromatosis or neurofibromatosis type 1 (NF1) is one of the most common autosomal-dominant disorders in man, with an incidence of approximately 1 in 3,000. The disease is characterized by multiple café-au-lait spots, cutaneous neurofibromas, Lisch nodules, and axillary freckling [Huson and Hughes, 1994]. The NF1 gene maps to 17q11.2 and was cloned by positional cloning [Wallace et al., 1991; Viskochil et al., 1990; Cawthon et al., 1990]. The gene spans approximately 350 kb of genomic DNA, and encodes a mRNA of 12 kb containing 59 exons and a 3.2-kb untranslated region [Li et al., 1995]. Four alternatively-spliced NF1 transcripts have been identified, and these are differentially expressed in different tissues.

The NF1 gene product called neurofibromin consists of 2,818 amino acids and demonstrates a significant homology to human GTPase-activating protein [Xu et al., 1990].

The mutation rate in the NF1 gene is approximately 10-fold higher than that reported for a single locus in man, and approximately 30–50% of all NF1 patients represent a new mutation. Approximately 150 mutations have so far been reported in the NF1 consortium. The great majority of NF1-specific mutations are predicted to result in a truncation of neurofibromin [Upadhyaya et al., 1994]. We report mutations in exon 37 of the NF1 gene in 6 unrelated individuals; of these, three mutations are novel.

The study group consisted of 320 unrelated NF1 patients and 50 normal controls. Genomic DNA was amplified using γ^{33}P-dATP end-labelled primers. Primer sequences were designated 5′ TCATTCCGAGATTCAGTTTAG 3′) and B (5′ AAAGTAACATTCAACACTGATA 3′). PCR was performed in a 6.5-μl reaction volume with 5 ng genomic DNA, 20 pmol of each primer, 200 μM dNTPs, 50 mmol/l KCl, 10 mmol/l Tris, pH 8.3, 1.5 mmol $MgCl_2$, 0.01% gelatin, and 1 unit Taq polymerase (Amersham, UK). The annealing temperature was 56°C. The 239-bp PCR product was diluted 10-fold and resolved on 0.5 × MDE gel for 18 hr at 7 watts, with and without 5% glycerol.

Sequencing of the variant SSCP (single-strand conformation polymorphism) was carried out on a purified PCR product (GeneClean, Stratech Scientific, UK) using the dideoxy chain termination method (Sequenase version 2.0, United States Biochemicals). Sequenase reactions were analyzed on a 6% denaturing sequence gel, dried onto filter paper, and exposed for 24–48 hr to X-ray film (Fuji) at room temperature.

For DNA analysis, RFLP 5′ to the NF1 gene [Ainsworth and Rodenhiser, 1991] and intron 27 and 38 microsatellites were used [Lazaro et al., 1993, 1994].

The specific mutations in 6 unrelated individuals identified by SSCP (Fig. 1) are summarized in Table I. All patients fulfilled the diagnostic criterion for NF1. The nonsense mutation G2281X occurs at nucleotide position 6841 and changes a CAG (glycine) to a TAG

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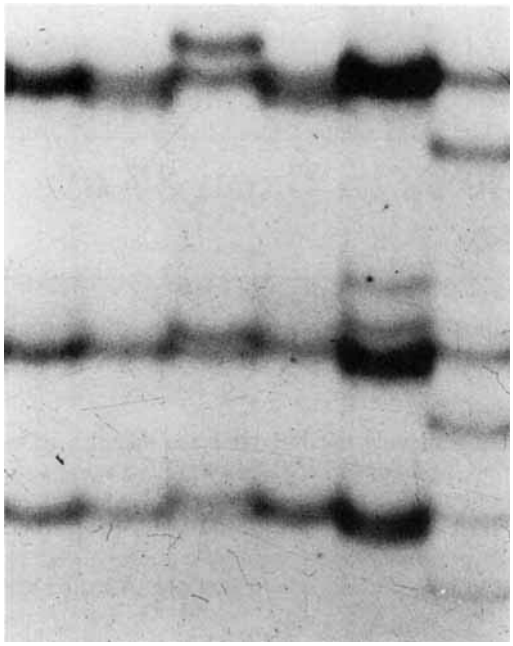

Fig. 1. SSCP variant bands identified in NF1 patients in exon 37. Genomic DNA (5 ng) was amplified using end-labelled primers (γ^{33} p) dATP. Radiolabelled PCR products were resolved on 0.5 × MDE gel. **Lanes 1–6** contain DNA samples from normal control, N490, Ns116, N203, N210, and N224, respectively.

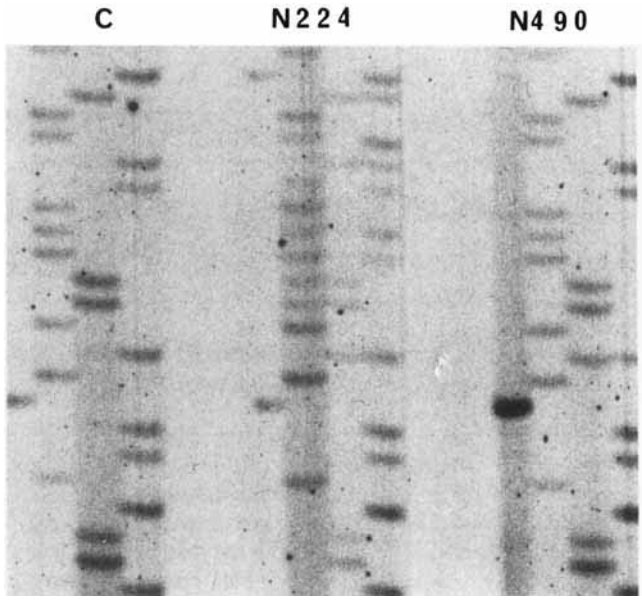

Fig. 2. Sequence analysis of patient N224. The deletion of TAC at nucleotide 6839 results in a stop codon. Sequence analysis of patient N490. A change from C to T results in the alteration of glycine (CAG) to a stop codon (TAG) at nucleotide 6841.

(stop codon) in patient N490 (Fig. 2). This alteration is predicted to generate a truncated protein with only 2,280 amino acids instead of the normal 2,818. This mutation was identified in patients N203 and N490, and both represent new mutation. Patient N490 has classical NF1 features and also has 2 affected children with mild learning difficulties. Patient N203 has multiple café-au-lait spots, neurofibromas, and Bell's palsy.

The nonsense mutation Y2264X identified in patients N210 and N828 has been previously reported in 2 unrelated NF1 patients [Robinson et al., 1995]. One of these patients has typical NF1, whereas clinical details on the others are not available.

The 1-bp insertion 6791 ins A resulted in a change of TAG (tyrosine) to a TAA (stop codon) in patient NS116. This change creates an extra *Mse*I site. This patient has skin involvement and severe scoliosis.

In patient N224, deletion of TAC at nucleotide 6839 generated a shift in the reading frame, resulting in the introduction of a stop codon (TAG) at codon 2280 (Fig. 2). This change creates a restriction site for enzyme *Tsp*509I. In addition to cutaneous involvement, this patient has hypertension, retinopathy, and left renal artery stenosis.

Family members of the patients with recurrent mutations were not available for analysis. To search for the origin of these mutations, DNA analysis with marker pHHH202 and the microsatellites for introns 27b and 38 demonstrated that patients N210, N828, N203, and N490 have different genotypes.

A nonsense mutation, R1947X, has been reported in 8 unrelated NF1 individuals, giving a frequency of approximately 2% [Cawthon et al., 1990; Estivill et al., 1991; Ainsworth et al., 1993; Horiuchi et al., 1994; Valero et al., 1994; Lazaro et al., 1995]. This alteration involves CpG dinucleotides, which show a high mutation rate in the human genome due to spontaneous deamination to thymine [Cooper and Youssoufian, 1988]. The

TABLE I. Details of Characterized Mutations in Exon 37 of NF1 Gene

Patient ID	Age (years)	Nucleotide change	Nucleotide position	Amino acid change	Restriction site	Effect on predicted protein	Type of NF1
N203	36	CAG to TAG	6841	G2281X	None	Truncated (2281)	Sporadic
N490	40	CAG to TAG	6841	G2281X	None	Truncated (2281)	Sporadic
N210	80	TAC to TAA	6792	Y2264X	Creates site for *Mse*I	Truncated (2264)	Sporadic
N828		TAC to TAA	6792	Y2264X	Creates site for *Mse*I	Truncated (2264)	Sporadic
Ns 116	31	A insertion TAC to TAA	6791-2	Tyr-stop	Creates site for *Mse*I	Truncated (2264)	Familial
N224	39	TAC deletion	6839	Leu-stop	Creates site for *Tsp*509 I	Truncated (2280)	Sporadic

nonsense mutations reported in this study do not involve CpG dinucleotides.

All changes identified in exon 37 in 6 unrelated NF1 patients occurred within a contiguous 49-bp sequence. The three previously published mutations Y2764X X 2, and 6789 del TTAC [Robinson et al., 1995], and the two unpublished changes 6790 ins TT and 6791 ins A (Wallace and Robinson, personal communication), are also located within this 49-bp region. Particular sequence patterns in the human genome are often associated with the preferential formation of insertions and deletions. From preliminary examination of the local sequence environment, no direct or inverted repeats were discernible.

Examination of the current data along with the previously published data identified two regions in exon 37 which appear to be highly mutable, the nucleotides at positions 6791–6792 and the nucleotides at position 6841. Although the functional aspects of exon 37 are not currently known, the identification of 11 independent disease-causing mutations in approximately 500 individuals within a contiguous 49-bp sequence would suggest that this region of the gene has as-yet unknown functional importance.

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